

STIC-ILL

mic

**From:** Ramirez, Delia  
**Sent:** Friday, August 24, 2001 5:16 PM  
**To:** STIC-ILL  
**Subject:** ILL order

Art Unit=1652  
Telephone=306-0288  
Application number=09/784340

Hi,

Please ignore the previous ILL order I sent with these references. I accidentally pressed the send button. This is the complete information. I would like to request photocopies of the following material:

Author (if known) <Jedlitschky et al >  
Article Title < >  
Journal or Book Title <Biochem. J.:>  
Volume And Issue if a Journal < 340>  
Pages if a Journal < 837-843 >  
Year Of Publication < 1999>

Author (if known) <Jin et al >  
Article Title < >  
Journal or Book Title <Biochem. Biophys. Res. Commun.>  
Volume And Issue if a Journal < 194>  
Pages if a Journal < 496-503>  
Year Of Publication < 1993>

Author (if known) <Lazard et al>  
Article Title < >  
Journal or Book Title <Nature:>  
Volume And Issue if a Journal < 349>  
Pages if a Journal < 790-793 >  
Year Of Publication < 1991>

Thanks,

---

Delia M. Ramirez, Ph.D.  
Patent Examiner - Art Unit 1652  
USPTO  
1911 S. Clark Street, Crystal Mall 1, 4E18  
Arlington, VA 22202  
(703) 306-0288  
delia.ramirez@uspto.gov

components, again either because of damage directly to modality-specific lexical representations or because of damage to access of these representations. The implication of this conclusion is that phonological and orthographic output representations are organized by grammatical category.

(3) Finally, the facts in (1) and (2) and the fact that H.W. and S.J.D. were impaired in processing only the verb form of homonymic words imply that the deficit does not concern specific lexical forms (for example, the orthographic form *crack*) but the grammatical category verb (of which *crack* is an instance) for modality-specific lexical forms. One implication of this latter conclusion is that we should give serious consideration to the possibility that grammatical category information is represented separately and redundantly in each modality-specific

lexical system.

In summary, taken together with recent results of category-specific deficits, the results we have reported suggest a remarkably specific organization of lexical knowledge in the brain, both at the semantic<sup>8</sup> and at the lexical form levels. Although at this time we do not have clear hypotheses about the nature of the brain mechanisms that compute lexical structure, it is clear that the information computed by these mechanisms must represent not only the phonological and orthographic form of words but also their grammatical class. The results reported for H.W. and S.J.D. pose a serious challenge for those models of lexical processing that would dispense with linguistic level information in the representation of lexical knowledge.<sup>29</sup> □

Received 13 September; accepted 31 December 1990.

1. Caramazza, A. A. *Risk Neurosci.* **11**, 395-421 (1988).
2. Shallice, T. *From Neuropsychology to Mental Structure* (Cambridge University Press, 1988).
3. Ellis, A. & Young, A. *Human Cognitive Neuropsychology* (Lawrence Erlbaum, 1988).
4. Warrington, E. K. Q. *J. exp. Psych.* **27**, 635-657 (1978).
5. Warrington, E. K. *Phil. Trans. R. Soc. Lond.* **B298**, 411-423 (1981).
6. Warrington, E. K. & Shallice, T. *Brain* **107**, 829-853 (1984).
7. Warrington, E. K. & McCarthy, R. A. *Brain* **106**, 859-878 (1983).
8. Warrington, E. K. & McCarthy, R. A. *Brain* **110**, 1273-1296 (1987).
9. McCarthy, R. A. & Warrington, E. K. *Nature* **324**, 428-430 (1989).
10. Basso, A., Capitani, E. & Lalonde, M. *J. Neurol. Neurosurg. Psychiatr.* **51**, 1201-1207 (1988).
11. Goodglass, H. & Budin, C. *Neuropsychologia* **26**, 67-78 (1988).
12. Sertori, G. & Job, R. *Cognitive Neuropsych.* **5**, 105-132 (1988).
13. Silver, M. C. & Galotti, G. *Cognitive Neuropsych.* **5**, 677-709 (1988).
14. Hills, A. & Caramazza, A. *Brain* (in the press).
15. Hart, J., Berndt, R. & Caramazza, A. *Nature* **318**, 338 (1985).
16. Semenza, C. & Zettin, M. *Nature* **342**, 678-679 (1989).
17. McKenna, P. & Warrington, E. K. *J. Neurol. Neurosurg. Psychiatr.* **41**, 571-573 (1978).

18. Miceli, G., Silver, M. C., Villa, G. & Caramazza, A. *Cortex* **20**, 217-220 (1984).
19. Baxter, D. & Warrington, E. K. *Neuropsychologia* **23**, 653-668 (1985).
20. McCarthy, R. A. & Warrington, E. K. *Neuropsychologia* **23**, 709-723 (1985).
21. Zingeser, L. B. & Berndt, R. S. *Cognitive Neuropsych.* **5**, 473-516 (1988).
22. Dunn, L. M. & Dunn, L. M. *Peabody Picture Vocabulary Test-Revised* (American Guidance Service, 1981).
23. Goodglass, H., Kaplan, E. & Weintraub, S. *The Revised Boston Naming Test* (Lee & Febiger, 1983).
24. Caramazza, A. & Hills, A. *Cortex* **26**, 95-122 (1990).
25. Bodecker, W. & Caramazza, A. *Cognitive Neuropsych.* (in the press).
26. Coltheart, M., Patterson, K. E. & Marshall, J. C. *Deep Dyslexia* (Routledge, 1980).
27. Allport, D. A. & Funnell, E. *Phil. Trans. R. Soc. Lond.* **B298**, 397-410 (1981).
28. Patterson, K. E. Q. *J. exp. Psych.* **30**, 587-601 (1978).
29. McClelland, J. L., Rumelhart, D. M. & Hinton, G. E. In *Parallel Distributed Processing: Explorations in the Microstructure of Cognition* Vol. 1 (eds McClelland, J. L. & Rumelhart, D. E.) 3-44 (MIT, 1986).

ACKNOWLEDGEMENTS: We thank H.W. and S.J.D. for their participation, and B. Bodecker, H. Egeth, M. McCloskey and B. Rapp for comments on this paper. The work was supported by NIMH, the Searer Institute, and the McDonnell-Pew Program in Cognitive Neuroscience.

## Odorant signal termination by olfactory UDP glucuronosyl transferase

Daniel Lazard, Karlin Zupko, Yitzhak Porla, Patrick Nef, Janette Lazarovits, Shirley Horn, Miriam Khen & Doron Lancet\*

Department of Membrane Research, The Weizmann Institute of Science, Rehovot, 76100 Israel

THE onset of olfactory transduction has been extensively studied<sup>1-7</sup>, but considerably less is known about the molecular basis of olfactory signal termination<sup>8,9</sup>. It has been suggested that the highly active cytochrome P<sub>450</sub> monooxygenases of olfactory neuroepithelium<sup>10-12</sup> are termination enzymes<sup>8,9,11,12</sup>, a notion supported by the identification and molecular cloning of olfactory-specific cytochrome P<sub>450</sub>s (refs. 13-16). But as reactions catalysed by cytochrome P<sub>450</sub> (refs. 17, 18) often do not significantly alter volatility, lipophilicity or odour properties<sup>8,11</sup>, cytochrome P<sub>450</sub> may not be solely responsible for olfactory signal termination. In liver and other tissues, drug hydroxylation by cytochrome P<sub>450</sub> is frequently followed by phase II biotransformation, for example by UDP glucuronosyl transferase (UGT), resulting in a major change of solubility and chemical properties<sup>19</sup>. We report here the molecular cloning and expression of an olfactory-specific UGT. The olfactory enzyme, but not the one in liver microsomes, shows preference for odorants over standard UGT substrates. Furthermore, glucuronic acid conjugation abolishes the ability of odorants<sup>1,20</sup> to stimulate olfactory adenyl cyclase. This, together with the known broad spectrum of drug-detoxification enzymes<sup>17,19</sup>, supports a role for olfactory UGT in terminating diverse odorant signals.

We previously obtained peptide sequences of bovine gp56, an olfactory epithelial-specific transmembrane glycoprotein

which is similar to UGT enzymes<sup>16</sup>. Based on the sequence information, a partial bovine complementary DNA clone (no. 21) encoding this protein was isolated. Using clone 21 as a probe, we isolated a full-length rat olfactory UGT clone (RO1) from a rat olfactory cDNA library (Fig. 1). The UGT clone shares several structural features with other UGT sequences (Fig. 1b). It is 87% identical to the partial bovine sequence (a possible orthologue), but only 44-60% identical to other rat liver UGT sequences (Fig. 1b). This, together with the expression results below, shows that a novel functional form, termed UGT<sub>olf</sub>, has been identified which defines a new subfamily within the UGT superfamily<sup>21</sup>.

As several olfactory signal-processing enzymes are tissue-specific<sup>4,7,22</sup>, we examined the tissue specificity of clone RO1. Figure 2 shows that cross-hybridizing messenger RNA is exclusively expressed in olfactory epithelium, with no reactivity seen in liver or other UGT-containing tissues. A similar tissue specificity has been observed for protein gp56 using immunoblot analysis<sup>16</sup>.

The tissue-specific expression of UGT<sub>olf</sub> points to a possible role in olfaction. By analogy with UGT function in liver<sup>19</sup>, olfactory UGT could help clear lipophilic odorant molecules from the chemosensory epithelium. We therefore asked whether olfactory UGT could glucuronidate odorants. When transiently expressed in COS-7 cells, rat UGT<sub>olf</sub> catalysed the glucuronidation of numerous, chemically diverse substrates, with several odorants showing particularly high efficiency (Fig. 3a). In parallel, we measured the enzyme activity in microsomal membranes from olfactory epithelium. Several established<sup>23</sup> (hydroxyl-containing) odorants were efficient UGT substrates (10-70 nmol per mg protein per min) in preparations from cow<sup>24</sup> (Fig. 3b) and rat (data not shown). The activity profile of UGT<sub>olf</sub> expressed in COS cells (Fig. 3a) is similar to that of the olfactory microsomal enzyme (Fig. 3b). Thus, UGT<sub>olf</sub> is probably a dominant component of the overall UGT activity in the olfactory epithelium. For all five odorants shown, the olfactory enzyme was two- to fivefold more active than the liver enzyme. In contrast, glucuronation of classical UGT substrates was comparable in both tissues (Fig. 3b).

\* To whom correspondence should be addressed.



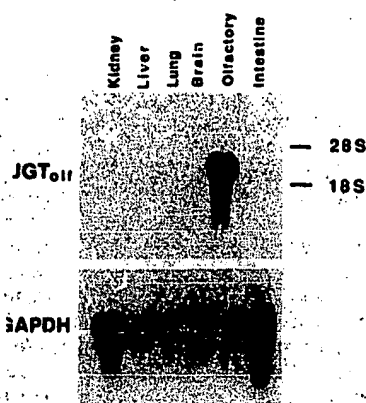


FIG. 2 Distribution of UGT<sub>off</sub> mRNA. Messenger RNAs from several rat tissues were probed with rat UGT<sub>off</sub> and with a control glyceraldehyde phosphate dehydrogenase (GAPDH) probe. No detectable signal is seen in tissues other than olfactory epithelium, even with long exposure times. The apparent length of the mRNA is ~2.5 kb. A similar analysis for the bovine UGT clone (not shown) corroborates this observation, except that respiratory epithelium (an adjacent, non-sensory nasal tissue) is also positive, but has a 3–4-fold lower reactivity. Positions of 28S and 18S rRNA are marked.

METHODS. Rat tissues were dissected in the laboratory immediately after death and frozen in liquid nitrogen. Total RNA (20 µg) prepared as described<sup>13</sup> was electrophoresed on 1% agarose gels and blotted onto nylon membranes (Genescreen, DuPont). Membranes were hybridized with <sup>32</sup>P-labelled cDNA of clone R01 (top) or mouse GAPDH (gift of Dr I. Ginsburg, the Weizmann Institute) (bottom). Hybridization was performed in Denhardt's solution with 1 M NaCl at 42 °C for 18 h, followed by two washes at 60 °C, 0.1 × SSC, for 30 min. Autoradiography was for 12 h at –70 °C.

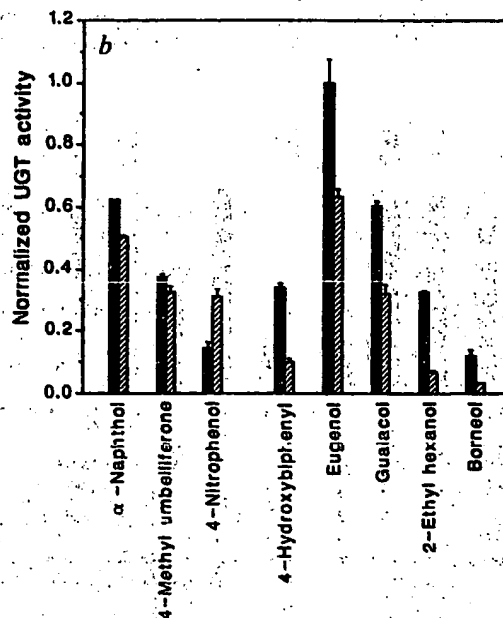
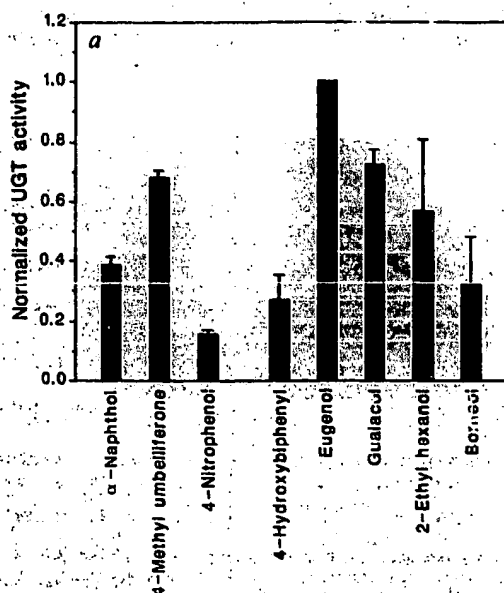
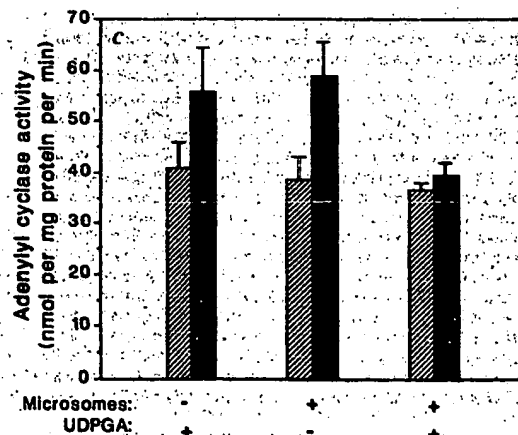


FIG. 3 Action and specificity of UGT<sub>off</sub>. a, Expression of rat UGT<sub>off</sub> in COS-7 cells. UGT activity towards standard substrates (three left-hand bars) and hydroxyl-containing odorants (five right-hand bars) was measured in homogenates of COS-7 cells transiently transfected with clone R01 cDNA. No glucuronidation could be detected in membranes from untransfected cells or from cells transfected with the cDNA in the wrong orientation. Values are normalized relative to eugenol, and represent the mean ± difference of two experiments with two batches of transfected cells. Specific activities towards eugenol were 1.33 and 0.29 nmol per mg protein per min, and in untransfected cells were less than 0.008 nmol per mg protein per min. b, UGT activity towards the same substrates in bovine olfactory epithelial (dark) and liver (hatched) microsomes. Values (normalized as in a) are representative of two experiments, and each is the mean ± difference of two replicates. Specific activity towards eugenol was 72.7 ± 5.5 nmol per mg protein per min. c, Glucuronation abolishes odorant activation of olfactory adenylyl cyclase (mean ± s.d. of triplicates;  $P < 0.02$ ). Odorants (or the vehicle alone for measurement of basal activity) were preincubated with or without bovine olfactory epithelial microsomes or UDP-glucuronic acid as indicated and subsequently used in the odorant-sensitive adenylyl cyclase assay<sup>1</sup>. □, Basal; ■, odorants.

METHODS. a, The R01 cDNA was inserted in two possible orientations in the mammalian expression vector pSVL-RS (provided by Dr C. Kahana, the Weizmann Institute), a modified pSVL vector (Pharmacia) in which an EcoRI site was introduced into the multiple cloning site. Subconfluent cultures of COS-7 cells were transfected with 0.24 µg recombinant plasmid per cm<sup>2</sup> as described<sup>23,26</sup>. After 72 h cells were ground-glass hand-homogenized and UGT activity assayed as described<sup>37</sup>, with modifications: incubation was with 0.1 M Tris-HCl, pH 7.4, 10 mM MgCl<sub>2</sub>, 0.05% Triton X-100, 1 mM glycerol, 50 µM UDP-glucuronic acid, 0.1 µCi <sup>14</sup>C-labelled UDP-glucuronic acid (304 mCi mmol<sup>-1</sup>; DuPont), and 10–60 µg homogenate protein in a total volume of 50 µl. After 2 h at 37 °C, the reaction was stopped with 150 µl ethanol, and samples analysed by thin-layer chromatography on silica gel as described<sup>37</sup>. b, Microsomes from bovine olfactory epithelium and liver were prepared as described<sup>26</sup>. UGT activity was assayed as described in a, except that the incubation mixture contained 1 mM UDP-glucuronic acid, 0.05 µCi <sup>14</sup>C-labelled UDP-glucuronic acid and 25 µg microsomal protein. Incubation time was 10 min and the reaction stopped with 3% SDS. Measure-



ments were carried out in the excess substrate regimen, and glucuronates identified with β-glucuronidase<sup>37</sup>. c, A mixture of the four hydroxyl-containing odorants, eugenol, gualacul, geraniol and 2-ethyl-1-hexanol (Aldrich, 62.5 µM each), was subjected to glucuronidation as in b, with the following modification: 100 µl reaction volume, 12 mM MgCl<sub>2</sub>, no Triton X-100, 100 µg microsomal protein, 3 mM UDP-glucuronic acid, and 30 min incubation time, selected to maximize odorant glucuronidation. The reaction mixture was centrifuged (5 min, 12,000g) and 20 µl supernatant together with the adenylyl cyclase reaction mix and rat olfactory cilia (0.5–1.0 µg protein) were analysed for cAMP generation as described<sup>1,38</sup>.

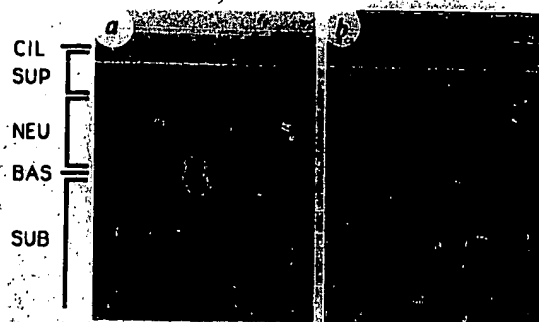


FIG. 4. Tissue localization of UGT<sub>off</sub>. Serial frozen sections of bovine olfactory epithelium were stained with a, haematoxylin-eosin or b, with an antiserum against UGT<sub>off</sub>. CIL, ciliary layer; SUP, supporting cells' apical cytoplasm and nuclei; NEU, sensory neuron nuclear layer; BAS, basal cell layer; SUB, subepithelial layers containing the Bowman's glands. b, Label is seen in the Bowman's glands (bg) and in a superficial layer (s). Labelling in the latter was more variable in location and intensity. No reactivity with olfactory epithelium was observed using non-specific rabbit serum, or an antiserum against an unrelated synthetic peptide, both purified as described below for the specific antiserum. The bar labelled SUP is 20  $\mu$ m.

**METHODS.** Bovine olfactory epithelia were collected as in Fig. 3 and stored in isopentane at  $-80^{\circ}\text{C}$ . Sections (8–10  $\mu$ m) were prepared on a Reichert-Jung Frigocut 2800 cryostat at  $-20^{\circ}\text{C}$ , dried overnight at  $23^{\circ}\text{C}$ , fixed for 20 min in acetone at  $-20^{\circ}\text{C}$  and washed in sodium phosphate buffer, pH 7.4, containing 0.15 M NaCl. Immunolabelling was performed with antiserum 11, prepared as described<sup>18</sup>, against a BSA-conjugated synthetic peptide corresponding to residues 229–244 of bovine UGT<sub>off</sub>. An immunoglobulin fraction was obtained through DEAE-cellulose (Whatman) ion-exchange chromatography, and anti-BSA reactivity was removed by three passes of affinity chromatography on a BSA-Sepharose column (Pharmacia), following which radioimmunoassays for BSA were negative. Sections were incubated with concentrated purified antiserum 11 (0.4 mg protein ml<sup>-1</sup>), and stained with tetramethylrhodamine-labelled goat anti-rabbit immunoglobulin antibody (gift of Dr B. Geiger, the Weizmann Institute).

cytochrome P<sub>450</sub> and UGT may be long-term detoxification devices. Olfactory epithelium is the region of the nervous system most exposed to airborne hazardous chemicals, and is only a few millimetres from the brain. Olfactory epithelium has been proposed to be a direct route of pathological agents into the brain<sup>30</sup> and is related to early changes associated with Alzheimer's disease<sup>31</sup>. The study of olfactory cytochrome P<sub>450</sub>s and UGT could shed light on a potentially relevant toxin inactivation mechanism. In cases where cytochrome P<sub>450</sub> action leads to cytotoxin bioactivation<sup>17,18</sup>, a highly active UGT may be the main detoxification barrier. □

Received 4 October; accepted 27 November 1990.

- Pace, U., Hanski, E., Salomon, Y. & Lancel, D. *Nature* **316**, 255–258 (1985).
- Sklar, P. B., Anhalt, R. R. & Snyder, S. H. *J. biol. Chem.* **261**, 15538–15543 (1986).
- Shirley, S. G., Robinson, C. J., Dickinson, K., Aupa, R. & Dodd, G. H. *Biochem. J.* **240**, 605–607 (1986).
- Lancel, D. & Pace, U. *Trends biochem. Sci.* **12**, 63–66 (1987).
- Lancel, D., Lazard, D., Heldman, J., Khen, M. & Neff, P. *Cold Spring Harbor Symp. Quant. Biol.* **53**, 343–348 (1988).
- Snyder, S. H., Sklar, P. B., Hwang, P. M. & Pevsner, J. *Trends Neurosci.* **12**, 35–38 (1989).
- Reed, R. R. *Cell* **60**, 1–2 (1990).
- Getchell, T. V., Margolis, F. L. & Getchell, M. L. *Prog. Neurobiol.* **23**, 317–345 (1984).
- Lancel, D. *A. Rev. Neurosci.* **9**, 329–355 (1986).
- Dahl, A. R., Hadley, W. M., Hahn, F. F., Benson, J. M. & McClellan, R. O. *Science* **218**, 57–59 (1982).
- Dahl, A. R. in *Molecular Neurobiology of the Olfactory System* (eds Margolis, F. L. & Getchell, T. V.) (Plenum, New York, 1988).
- Jenner, J. & Dodd, G. H. *Drug Metabol. Drug Interact.* **6**, 123–148 (1988).
- Neff, P. *et al. J. biol. Chem.* **264**, 6780–6785 (1989).
- Ding, X. X. & Coon, M. J. *Biochemistry* **27**, 8330–8337 (1988).
- Neff, P., Larabee, T. M., Nagimoto, K. & Meyer, U. A. *J. biol. Chem.* **265**, 2903–2907 (1990).
- Lazard, D. *et al. Biochemistry* **29**, 7433–7440 (1990).
- Black, S. D. & Coon, M. J. *Adv. Enzym.* **60**, 35–67 (1987).
- Nebert, D. W. & Gonzalez, F. J. *A. Rev. Biochem.* **56**, 945–993 (1987).
- Burchell, B. & Coughlin, M. W. *Pharmac. Ther.* **43**, 261–289 (1989).
- Pace, U. & Lancel, D. *Proc. natn. Acad. Sci. U.S.A.* **83**, 4947–4951 (1986).
- Mackenzie, P. I., Choudhury, N. R. & Chowdhury, J. R. *Clin. exp. pharmacol. Physiol.* **16**, 501–504 (1989).
- Pfeuffer, E., Molter, J., Lancel, D. & Pfeuffer, T. *J. biol. Chem.* **264**, 18803–18807 (1989).
- Fenaroli, G. *Fenaroli, G. Handbook of Flavor Ingredients* (eds Furia, E. T. & Bellanca, N.) (CRC, Cleveland, 1975).
- Lazard, D., Barak, Y. & Lancel, D. *Biochem. biophys. Acta* **1013**, 68–72 (1989).
- Voigt, J. M., Guengerich, F. P. & Baron, J. *Cancer Lett.* **27**, 241–247 (1985).
- Foster, J. R. *et al. Biochem. Pharmacol.* **36**, 4543–4554 (1986).
- Shepherd, S. R., Baird, S. J., Hallinan, T. & Burchell, B. *Biochem. J.* **259**, 617–620 (1989).
- Crawford, J. M. & Goltan, J. L. *Am. J. Physiol.* **256**, 121–131 (1988).
- Gander, J. E. & Manning, G. J. *Pharmac. Ther.* **10**, 191–221 (1980).
- Shiple, M. T. *Brain Res. Bull.* **15**, 129–142 (1985).
- Talano, B. R. *et al. Nature* **337**, 736–739 (1989).
- Wilbur, W. & Lipman, D. *Proc. natn. Acad. Sci. U.S.A.* **80**, 726–730 (1983).
- Mackenzie, P. I. *J. biol. Chem.* **261**, 6119–6125 (1986).
- Mackenzie, P. I. *J. biol. Chem.* **262**, 9744–9749 (1987).
- Sompayrac, L. M. & Darna, K. J. *Proc. natn. Acad. Sci. U.S.A.* **78**, 7575–7578 (1981).
- Graham, F. L. & Van der Eb, A. J. *Virology* **52**, 456–467 (1973).
- Bansal, S. K. & Gessner, T. *Analyt. Biochem.* **109**, 321–329 (1980).
- Salomon, Y., Lodos, C. & Rodbell, M. *Analyt. Biochem.* **68**, 541–548 (1974).

**ACKNOWLEDGEMENTS.** We thank Drs D. W. Nebert, C. Kahana, Y. Rosenberg-Nasson and Y. Barak for help and advice. This work was supported by the National Institutes of Health, the United States Army Research Office, the Fund for Basic Research of the Israeli Academy of Sciences, the Heisenberg and Minerva Foundations, Munich, Germany, and the Fordthorner Center for Molecular Genetics.

## Activation of chloride channels in normal and cystic fibrosis airway epithelial cells by multifunctional calcium/calmodulin-dependent protein kinase

John A. Wagner<sup>†</sup>, Alison L. Cozen<sup>†</sup>, Howard Schuman<sup>§</sup>, Dieter C. Gruenert<sup>‡</sup>, Lubert Stryer<sup>†</sup> & Phyllis Gardner<sup>¶</sup>

<sup>\*</sup> Department of Medicine, Falk CVRC, Stanford University School of Medicine, Stanford, California 94305, USA

<sup>†</sup> Department of Cell Biology, Stanford University School of Medicine, Stanford, California 94305, USA

<sup>‡</sup> Cardiovascular Research Institute, University of California, San Francisco, California 94143, USA

<sup>§</sup> Department of Pharmacology, Stanford University School of Medicine, Stanford, California 94305, USA

**CYSTIC fibrosis** is associated with defective regulation of apical membrane chloride channels in airway epithelial cells. These channels in normal cells are activated by cyclic AMP-dependent protein kinase<sup>1,2</sup> and protein kinase C<sup>3,4</sup>. In cystic fibrosis these kinases fail to activate otherwise normal Cl<sup>-</sup> channels<sup>1–4</sup>. But Cl<sup>-</sup> flux in cystic fibrosis cells, as in normal cells, can be activated by raising intracellular Ca<sup>2+</sup> (refs 5–10). We report here whole-cell patch clamp studies of normal and cystic fibrosis-derived airway epithelial cells showing that Cl<sup>-</sup> channel activation by Ca<sup>2+</sup> is mediated by multifunctional Ca<sup>2+</sup>/calmodulin-dependent protein kinase. We find that intracellular application of activated kinase and ATP activates a Cl<sup>-</sup> current similar to that activated by a Ca<sup>2+</sup> ionophore, that peptide inhibitors of either the kinase or calmodulin block Ca<sup>2+</sup>-dependent activation of Cl<sup>-</sup> channels, and that a peptide inhibitor of protein kinase C does not block Ca<sup>2+</sup>-dependent activation. Ca<sup>2+</sup>/calmodulin activation of Cl<sup>-</sup> channels presents a pathway with therapeutic potential for circumventing defective regulation of Cl<sup>-</sup> channels in cystic fibrosis.

To delineate the molecular mechanism by which Ca<sup>2+</sup> activates Cl<sup>-</sup> channels, we used three simian virus 40 (SV40)-transformed cell lines from normal and cystic fibrosis (CF) airway epithelia<sup>11–13</sup>. All three contain messenger RNA for the cystic fibrosis transmembrane regulator (CFTR)<sup>14</sup>. One of the CF cell lines (2CFSMEo-; CF-1) was positive for the  $\Delta$ F508 mutation, the deletion in 70% of CF chromosomes<sup>15</sup>, whereas the other (CFNPE-9o-; CF-2) was not<sup>13</sup>.

Whole-cell patch clamp recording revealed that only the normal fetal cell line (56FHTe-8o-; refs 3, 20) showed increased Cl<sup>-</sup> conductance in response to bath application of 400  $\mu$ M

□ To whom correspondence should be addressed